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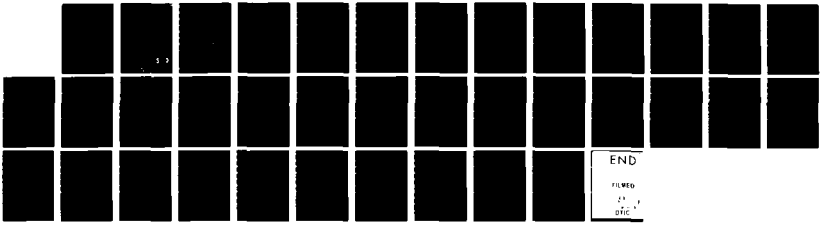

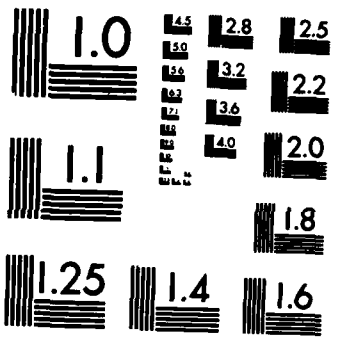

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DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
IV. A Blood Acid-Base Curve Nomogram for Immature Pigs

JOHN P. HANNON, PhD

DIVISION OF COMBAT CASUALTY CARE

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20. Abstract:

The normal acid-base characteristics of porcine blood have been poorly defined, largely because of procedural differences in animal handling, blood sampling, and measurement techniques. Consequently, 40 immature, 20- to 31-kg domestic pigs were used to establish population characteristics for arterial blood. Samples were collected from chronically implanted catheters while the animals were maintained under steady state, near-basal conditions. At a measurement temperature of 38 C, pH averaged 7.496; P CO_2, 40.6 torr; $[HCO_3^-]$, 31.6 mEq/l; P O_2, 79.1 torr; hemoglobin, 9.65 g/dl; hematocrit, 0.29; plasma albumin, 25.3 g/l; plasma globulin, 32.3 g/l; and plasma buffer base, 45.4 mEq/l. Hourly measurements over a 6-hour period in 6 of these pigs showed a small but significant decrease in P O_2 with time but no significant change in acid-base status. The data showed that nomograms or other procedures based on human blood characteristics were invalid when used to estimate base excess concentration of porcine blood. The normal pH of arterial blood was higher in pigs than in humans; hence, parameters defining zero base excess differed in the two species. Consequently, constant P CO_2 titrations were performed on arterial samples taken from 10 pigs and the data were used to construct an acid-base curve nomogram in which zero base excess was defined for blood with a pH of 7.50 and a P CO_2 of 40 torr. This nomogram was compared to a nomogram for human blood based on constant P CO_2 titrations of 3 human blood samples and zero base excess defined for a pH of 7.40 and a P CO_2 of 40 torr. The porcine curve was displaced to the right of the human curve. The pH/log P CO_2 coordinates defining base excess loci also differed in the two species. The higher plasma buffer base concentration of porcine blood, relative to that of human blood, conferred a greater capacity to compensate for acid loads. To assess the acid-base status of porcine blood accurately, therefore, a nomogram or other procedure appropriate to the characteristics of porcine blood should be used.

ABSTRACT

The normal acid-base characteristics of porcine blood have been poorly defined, largely because of procedural differences in animal handling, blood sampling, and measurement techniques. Consequently, 40 immature, 20- to 31-kg domestic pigs were used to establish population characteristics for arterial blood. Samples were collected from chronically implanted catheters while the animals were maintained under steady state, near-basal conditions. At a measurement temperature of 38 C, pH averaged 7.496; P CO_2, 40.6 torr; [HCO_3^-], 31.6 mEq/l; P O_2, 79.1 torr; hemoglobin, 9.65 g/dl; hematocrit, 0.29; plasma albumin, 25.3 g/l; plasma globulin, 32.3 g/l; and plasma buffer base, 45.4 mEq/l. Hourly measurements over a 6-hour period in 6 of these pigs showed a small but significant decrease in P O_2 with time but no significant change in acid-base status. The data showed that nomograms or other procedures based on human blood characteristics were invalid when used to estimate base excess concentration of porcine blood. The normal pH of arterial blood was higher in pigs than in humans; hence, parameters defining zero base excess differed in the two species. Consequently, constant P CO_2 titrations were performed on arterial samples taken from 10 pigs and the data were used to construct an acid-base curve nomogram in which zero base excess was defined for blood with a pH of 7.50 and a P CO_2 of 40 torr. This nomogram was compared to a nomogram for human blood based on constant P CO_2 titrations of 3 human blood samples and zero base excess defined for a pH of 7.40 and a P CO_2 of 40 torr. The porcine curve was displaced to the right of the human curve. The pH/log P CO_2 coordinates defining base excess loci also differed in the two species. The higher plasma buffer base concentration of porcine blood, relative to that of human blood, conferred a greater capacity to compensate for acid loads. To assess the acid-base status of porcine blood accurately, therefore, a nomogram or other procedure appropriate to the characteristics of porcine blood should be used.

Key Words: Acid-Base Nomogram, Blood, Swine

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DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
IV. A BLOOD ACID-BASE CURVE NOMOGRAM FOR IMMATURE PIGS

In a recent study of hemorrhagic hypotension in conscious domestic swine (1), control values for certain blood gas and acid-base variables differed substantially from those characteristic of humans and dogs. The normal pH of porcine arterial blood averaged about 7.50, a value distinctly higher than the 7.40 average usually reported in humans (2) and dogs (3) studied under equivalent conditions. Arterial P CO_2 values of the pig averaged about 40 torr, about the same as humans (2) but higher than the 33 or 34 torr recorded in dogs (3). The high arterial pH value in pigs was attributable to an elevated bicarbonate concentration, averaging about 31 mEq/l as compared to 24 or 25 mEq/l in humans (2), and 21 or 22 mEq/l in dogs (3). In addition, the normal arterial base excess of swine averaged about +7 mEq/l as compared to 0 in both humans and dogs (2,3). The elevated base excess in swine appeared attributable for the most part to a high bicarbonate concentration. Porcine data consistent with this interpretation have been reported by Scott and McIntosh (4). This interpretation, however, had to be tempered by the knowledge that the base excess estimates for swine were based on Siggaard-Andersen nomograms for human blood (5,6). The latter, by definition, assigns a zero value to base excess when blood pH is 7.40 and P CO_2 is 40 torr. Scott and McIntosh (4) considered the Siggaard-Andersen curve nomogram (5) applicable to porcine blood since acid additions produced appropriate changes in base excess. Their data, nevertheless, showed that an acid addition of about 8 mEq/l would be required to achieve a zero base excess value on the human nomogram. A divergent opinion regarding the applicability of the human curve nomogram to porcine blood was reported by Riordan et al (7). These investigators (7) actually constructed a porcine curve nomogram according to the Siggaard-Andersen procedure (5,8). In doing so, they assumed that zero base excess should be obtained when pH was 7.40 and P CO_2 was 40 torr. Clearly, if the pH of arterial blood from normal swine is about 7.50 when P CO_2 is 40 torr, this assumption would not only lead to construction of an invalid nomogram, but also use of such a nomogram would lead also to invalid data when base excess concentration was estimated under abnormal conditions. In the study reported here, a curve nomogram appropriate to porcine blood was constructed. Initially, population characteristics for the arterial blood gas and acid-base status of normal young domestic pigs were obtained under near-basal conditions. These data were used subsequently to establish parameters critical to nomogram construction.

METHODS

Young domestic pigs (Hampshire-Duroc Cross), both barrows and gilts, were used in the studies reported here. They were obtained from a commercial breeder (J.G. Boswell, Corcoran, CA) and were maintained in a common indoor holding area at Letterman Army Institute of Research until utilized for study, usually within two to four weeks after arrival. They were fed a commercial ration (Purina Pig Chow, Ralston Purina Co., St. Louis, MO) and received water ad libitum. At the time of this study they were between two and three months old and weighed 20-31 kg.

After an overnight fast, each pig was brought into the animal surgical suite and anesthetized. A preanesthetic injection of 0.08 mg/kg atropine, 2.2 mg/kg ketamine HCl, and 1.1 mg/kg xylazine HCl was followed by halothane administered by face mask. Under sterile conditions, a combination 1.02-mm ID x 2.16-mm OD medical grade Silastic^{R} tubing (Dow Corning, Midland, MI) joined to 1.27-mm ID x 2.03-mm OD type S45-HL Tygon^{R}, type S45-HL, tubing (Norton Plastics and Synthetics, Akron, OH) was inserted and secured in the carotid artery. The Silastic^{R} portion was placed intravascularly and the Tygon^{R} portion was tunneled beneath the skin and exited on the dorsal surface of the neck. The exteriorized portion was fitted with a 16-gauge Intramedic^{R} Luer Stub Adapter (Clay Adams, Parsippany, NJ) and capped with an Argyle^{R} Intermittent Infusion Plug (Brunswick Co., St. Louis, MO). Following wound closure, the catheter was filled through the infusion plug with heparinized saline (500 units/ml). The dorsal exit site was protected folowing surgery by a 5-cm x 10-cm Velcro^{R} patch (Velcro USA, New York, NY) sutured to the skin; a 2-cm x 7.5-cm hole was cut in the patch portion next to the skin to allow catheter access.

After a 7- to 10-day recovery period each pig, after an overnight fast, was brought into a quiet laboratory in a portable transport cage and was given a variety of fabric bedding material. After 15 to 30 minutes of rooting and bedding rearrangement, most animals voluntarily assumed a recumbent position. When so positioned, the intermittent infusion plug was removed and the stub adapter was connected to a 12-inch pressure monitoring/injection line (Cobe Laboratories, Lakewood, CO). The latter had been previously fitted with a plastic three-way stopcock (Pharmaseal^{R}, Inc., Toa Alta, Puerto Rico) and filled with heparinized saline (10 units/ml). The entire system was then cleansed by withdrawing 10 ml of fluid (blood plus heparinized saline) followed by flushing with fresh heparinized saline. After 30 or more minutes of additional voluntary recumbent rest, duplicate or triplicate blood samples were taken at 10-minute intervals for blood gas, acid-base, and related plasma biochemical measurements. A total of 40 pigs were so utilized to obtain the population characteristics for normal young animals. In six of these pigs, all measurements were repeated at hourly intervals for 6 hours to assess the extent, if any, of diurnal changes. The blood gas and acid-base measurements were made at 38 C with an Instrumentation Laboratory

Model 813 automated analyzer (Instrumentation Laboratory, Inc., Lexington, MA). Precision buffer solutions (pH 6.840 and 7.384) and analyzed gases (± 0.03%) prepared by Instrumentation Laboratory were used to calibrate the pH, P O_2, and P CO_2 electrodes. Hematocrit determinations were obtained with a Lourdes microhematocrit centrifuge (Vernitron Medical Products, Carlstadt, NJ) and hemoglobin determinations with an Instrumentations Laboratory Cooximeter. Dead space in the sampling syringes used to obtain blood samples was filled with heparin (1000 units/ml), and all blood gas and acid-base measurements were made immediately after blood withdrawals. The remaining blood was centrifuged and plasma collected for determination of protein anion concentration.

Ten additional pigs were used to obtain arterial blood for the construction of base-excess curve nomograms according to the Siggaard-Andersen procedure (5,8). The animals were surgically prepared and treated in all respects like those used in the population studies. Details of the constant P CO_2 titration procedures are described elsewhere (Appendix A). Briefly, a 60-ml blood sample was taken from each animal and chilled to 1 or 2 C in ice water. It was then lightly centrifuged to allow plasma collection and the preparation of an erythrocyte-plasma mixture (blood) with a hemoglobin concentration of 16-20 g/dl. A Lourdes microhematocrit centrifuge and a Fisher Model 740 digital hemophotometer (Fisher Scientific Co., Pittsburg, PA) and Drabkins reagent were used to determine the hematocrit and hemoglobin characteristics; standard procedures established by the instrument manufacturers were followed. Then, 1-ml aliquots of plasma or blood were accurately pipetted with a positive displacement SMI micropipette (Scientific Manufacturing Industries, Emeryville, CA) into 9 pairs of 12x75 mm test tubes, also placed in ice water. To each tube of a pair, 0.25 ml of hydrochloric acid was added, one tube at a time, to give a final base deficit of 0.20, 0.15, 10, 5, and 0 (saline) mEq/l or sodium bicarbonate to give a final base excess of 5, 10, 20, and 30 mEq/l. To avoid hemolysis, sufficient NaCl had been added to the acid and base solutions to give a final sodium concentration of 0.15 M. Furthermore, the test tubes were lightly centrifuged so that acid or base could be added slowly to plasma layer with litte or no hemolysis of the blood sample. After acid or base addition, the tube contents were mixed rapidly with a vortex mixer and transferred immediately to a Instrumentation Laboratory Model 237 tonometer where the sample was equilibrated for 5 minutes at 38 C with either 4 or 8 percent CO_2 in air. A temperature of 38 C, rather than 37 C, allowed data comparison with the work of other investigators (4-9). The gas mixtures were Matheson Primary Standard Grade (Matheson Co., Newark, CA) with certified (measured) concentrations of 3.992 and 7.986 percent CO_2. Preliminary determinations showed temperature and gas equilibration in the tonometer were achieved within 2 minutes. After equilibration, the plasma or blood sample was transferred directly to an Instrumentation Laboratory Model 113-S1 ultra-micro pH/blood gas system for pH determinations. About two and a half hours were required for the

processing of all samples from a given animal and, while awaiting tonometry, the test tubes containing plasma or blood samples were kept in ice water to minimize deterioration.

For comparative purposes, base excess curve nomograms were also constructed for human blood. Venous samples were thus obtained from three rested male volunteers and, subsequently, were subjected to identical treatment procedures as those used for porcine blood.

Total plasma protein (10) and albumin (11) were determined with a GEMSAEC centrifugal analyzer (Electro-Nucleonics, Fairfield, NJ). Globulin concentration was estimated as the difference between total protein and albumin concentrations. Concentrations in mEq/l plasma were calculated by the formulae of Van Slyke et al (12). Their formulae were modified as follows to allow utilization of grams of plasma protein rather than grams of protein nitrogen per liter in the calculations:

$$P_a(\text{mEq/l}) = 0.125(\text{g alb/l})(\text{pH}-5.16) + 0.077(\text{g glob/l})(\text{pH}-4.89)$$

where P_a refers to total plasma protein anion concentration in mEq/l of plasma.

In the population study, data were summarized in terms of the mean, range of values, standard deviation (S.D.), and standard error of the mean (S.E.M.). Other tabular data were summarized in terms of the mean $\pm$ S.D. or $\pm$ S.E.M. Where tested, statistical significance ($p \leq 0.05$) was evaluated with single factor analysis of variance.

RESULTS

Table 1 summarizes the arterial blood gas and acid-base status of the 40 pigs used to establish population characteristics typical of the conscious, unrestrained animal studied under near-basal condition. These data indicate that the young pig has higher values for plasma pH and bicarbonate concentration but lower values for blood P O_2, hematocrit, and hemoglobin concentrations, and for plasma albumin and globulin concentrations relative to those typically reported for humans and dogs. The plasma buffer base concentration, calculated as the sum of plasma bicarbonate and protein anion concentrations, exceeds the values usually seen in humans and dogs.

When the blood gas and acid-base measurements of the recumbent pig were repeated at hourly intervals during the course of a day (from early morning to mid-afternoon), little change in status was noted (Table 2). Only P O_2 showed a statistically significant change--a slight decrease.

Base excess data were not included in Tables 1 and 2 since the automated apparatus used in this study was programmed to compute base

excess values on the basis of pH and P CO_2 parameters defined for human blood. Porcine values so computed were always positive, averaging +7.7 mEq/l. These positive values showed a statistically significant decrease of about 2 mEq/l in the pigs measured at hourly intervals for 6 hours.

Figure 1 illustrates the composite porcine base excess curve nomogram which was constructed from titration data obtained from 10 arterial blood and plasma samples. Construction was based on the assignment of a zero base excess value to blood or plasma with a pH of 7.50 and a P CO_2 of 40 torr; these parameters were consistent with the porcine population data reported in Table 1. The standard bicarbonate scale included in Figure 1 was calculated from the Henderson-Hasselbalch equation, employing the pH-dependent pK' values for plasma reported by Severinghaus et al (13). Table 3 summarizes the pH and P CO_2 coordinate data which were used to establish the base deficit and base excess loci for curve construction. It is apparent from these data that the accuracy of loci positioning in the overall nomogram was greatest for titrations involving numerically low values, and least for numerically high values, of acid or base addition to plasma and blood; loci positioning was particularly variable with the larger additions of bicarbonate to the titration samples. Similar levels of variability were reported for human blood titrations by Siggaard-Andersen and Engel (8) and for canine blood titrations by Emuakpor et al (14).

Characteristics of the porcine curve nomogram relative to those of the human nomogram are compared in Figure 2. Titration data from three venous blood samples were used to construct the human curve. The two nomograms, although qualitatively similar, differ in two important, quantitative respects. First, the porcine curve is displaced to the right of the human curve, an effect that is entirely attributable to the pH coordinates which were selected to define zero base excess. A value of 7.50 was chosen for swine, 7.40 for humans. The second quantitative species difference concerned the pH and P CO_2 coordinates which defined the loci for comparable additions of acid or base. For human blood, these loci were essentially the same as those reported by Siggaard-Andersen (5). In porcine blood, however, acid additions produced less displacement down the left-hand arm of the curve than the same additions to human blood. Base additions, in contrast, produced greater displacement down the right-hand arm of the porcine as compared to the human curve.

Table 4 summarizes the effects of changes in base excess concentrations on the pH and standard bicarbonate concentration of porcine and human plasma. The pH values for a P CO_2 of 40 torr were obtained from the plasma titration curves as described by Siggaard-Andersen (5), and the bicarbonate concentrations associated with those pH values were computed by the Henderson-Hasselbalch equation. The data show that equivalent changes in the base excess concentration of porcine and human blood produced, as would be

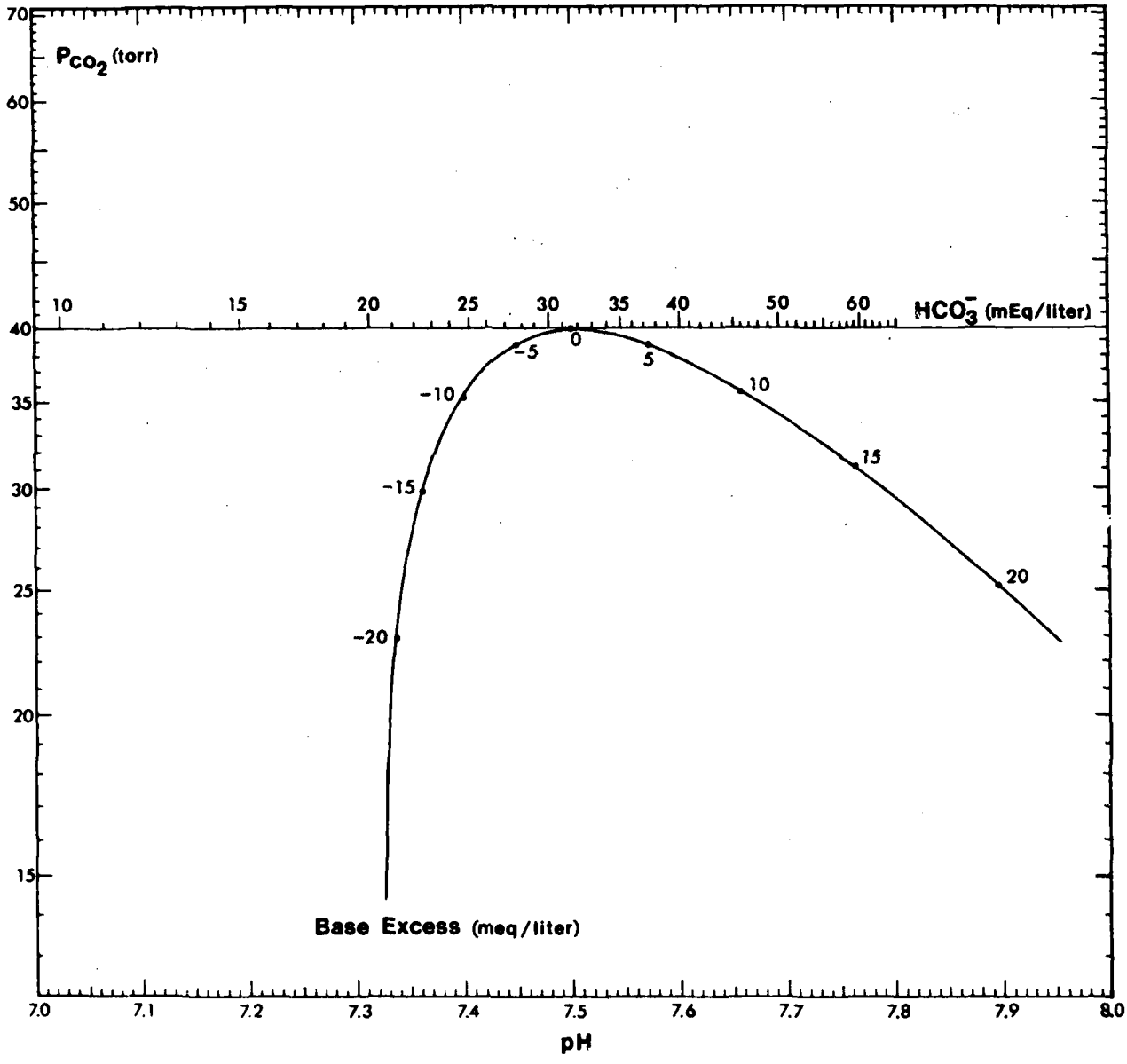

Figure 1. Base-excess curve nomogram for porcine blood.

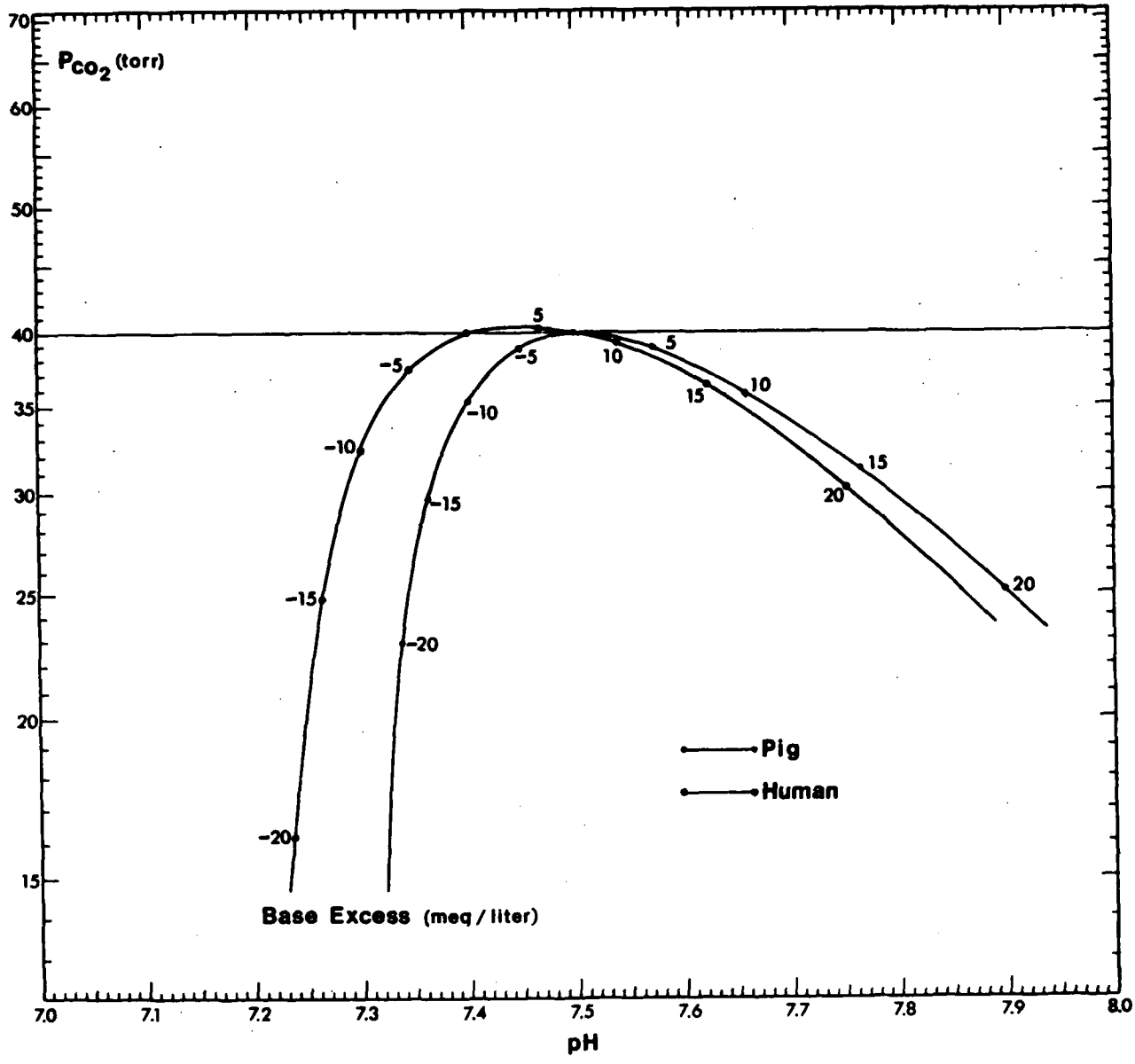

Figure 2. Comparison of composite base excess curve nomograms for human (N = 3) and porcine (N = 10) blood.

Table 1. Arterial blood gas and acid-base characteristics of conscious young domestic pigs (n=40) measured under near basal conditions.

Component	Mean	Range	S.D.	S.E.M.
Body weight (kg)	24.6	20.0-30.9	4.01	0.63
Hematocrit	0.29	0.24-0.34	0.0438	0.0069
Hemoglobin (g/dl)	9.65	8.7-11.1	0.67	0.11
P O_2 (torr)	79.1	69.2-85.9	4.05	0.64
pH	7.496	7.441-7.527	0.0183	0.0029
P CO_2 (torr)	40.6	36.5-46.0	2.56	0.40
Plasma $[HCO_3^-]$ (mEq/l)	31.6	26.9-36.4	2.29	0.36
Plasma albumin (g/l)	25.4	21.6-28.0	2.02	0.32
Plasma globulin (g/l)	32.2	27.1-41.2	4.43	0.70
Plasma buffer base (mEq/l)	45.4	39.8-51.6	3.25	0.51

Table 2. Arterial blood gas and acid-base characteristics of conscious young domestic pigs measured at hourly intervals under near basal conditions.

Time (Hrs)	P O_2 (torr)	pH	P CO_2 (torr)	$[HCO_3^-]_p$ (mEq/l)
0	83.1±0.75	7.499±0.0023	40.0±0.86	31.0±0.52
1	80.1±1.29	7.504±0.0065	39.4±1.27	30.6±0.47
2	79.2±1.92	7.502±0.0027	39.7±1.04	31.0±0.70
3	76.5±1.03	7.505±0.0037	38.6±1.06	30.3±0.52
4	78.2±1.19	7.498±0.0048	38.8±0.99	30.0±0.55
5	77.1±0.70	7.501±0.0039	38.3±0.91	29.8±0.54
6	78.6±1.08	7.493±0.0024	39.1±0.93	29.9±0.63
F-Ratio	5.39*	1.75	1.67	1.95

Values represent the mean ± SEM for 6 animals. The 0-hour value is the average of duplicate determinations made after 30-60 minutes of voluntary recumbency.

*Indicates significant change with time ($p \leq 0.05$): $F_{7,35}=2.29$, as determined by analysis of variance.

Table 3. Porcine curve nomogram: pH/P CO_2 coordinates as a function of base excess concentration

Base Excess (mEq/l)	Coordinates pH	P CO_2
-20	7.336±0.0083	22.9±0.93
-15	7.362±0.0084	29.8±0.88
-10	7.400±0.0068	35.2±0.90
- 5	7.446±0.0040	38.7±0.17
0	7.50	40
5	7.512±0.0039	38.9±0.47
10	7.656±0.0111	35.5±1.08
15	7.766±0.0201	31.3±1.62
20	7.897±0.0317	25.3±2.43

Values for pH/P CO_2 indicate mean ± S.D. for arterial blood from 10 pigs.

Table 4. Effect of base excess changes on the pH and standard bicarbonate concentration of porcine and human plasma

Base Excess	Porcine pH	Porcine $[HCO_3^-]$	Human pH	Human $[HCO_3^-]$
-20	7.141±0.0449	12.9±0.72	6.908±0.0592	7.7±1.01
-15	7.249±0.0142	17.2±0.57	7.093±0.0142	11.8±0.38
-10	7.349±0.0081	21.8±0.46	7.233±0.0026	16.5±0.10
- 5	7.431±0.0054	26.6±0.38	7.321±0.0041	20.5±0.17
0	7.500± 0	31.1± 0	7.400± 0	24.7± 0
5	7.561±0.0054	36.4±0.48	7.470±0.0011	29.4±0.10
10	7.613±0.0034	41.2±0.37	7.532±0.0021	34.0±0.15
15	7.662±0.0064	46.4±0.70	7.589±0.0036	39.3±0.32
20	7.706±0.0089	51.7±1.08	7.650±0.0116	44.0±0.04

Values indicate the mean ± S.D. of results from 10 porcine and 3 human blood titrations.

expected, nearly equivalent changes in standard bicarbonate concentration. The absolute values associated with a given base excess level, however, were always lower in human than in porcine plasma. This finding was due entirely to the higher standard bicarbonate concentration at zero base excess in porcine as compared to human plasma. In contrast to the foregoing, equivalent changes in base excess produced significantly greater changes in the pH of human plasma relative to porcine plasma. At a base excess of -20 mEq/l, for example, the pH of porcine plasma was reduced by 0.359 units from the zero base excess value, whereas an equivalent base change in human plasma produced a pH decrement of 0.492 units. Similar species differences, but to a lesser degree, were seen with positive base excess changes. Absolute pH level at a given base excess concentration was always higher in porcine plasma than in human plasma because of the species difference in pH at zero base excess.

DISCUSSION

It is readily evident from the data presented here that faulty values will be obtained if porcine base excess concentration is estimated by a nomogram or automated blood gas apparatus designed for use with human blood. In the normal resting pig, arterial values so obtained will be 7-8 mEq/l too high. The error is attributable to the higher resting pH and bicarbonate concentration of porcine as compared to human blood. If this were the only species difference, then reasonably accurate estimates of porcine base excess concentration could be obtained by subtracting 7 or 8 mEq/l from the human nomogram or automated apparatus value. Pig blood, however, has buffering characteristics which are distinctly different from those of human blood. These differences in the acidotic or alkalotic animal lead to base excess changes which deviate quantitatively and qualitatively from those seen in the acidotic or alkalotic human. Reliable estimates of porcine base excess concentration, therefore, can only be derived from a nomogram or other procedure specifically developed for use with porcine blood. In the construction of such a nomogram, zero base excess should be assigned to plasma or blood with a pH of 7.50 and a P CO_2 of 40 torr. Consequently, the nomogram for porcine blood reported by Riordan et al (7) would provide inaccurate assessments of acid-base status, because it assigns zero base excess to blood with a pH of 7.40 and a P CO_2 of 40 torr. For the same reason, applicability of the Siggaard-Andersen (5) human nomogram to porcine blood reported by Scott and McIntosh (4) also would be invalid.

The concentrations of buffer components in arterial blood obtained from the pig population used in this study revealed three major deviations from those commonly seen in human. First, the porcine blood had a lower hemoglobin concentration, averaging 9.65 g/dl as compared to the 12-16 g/dl typical of adult human blood (15). Total plasma protein concentration in pig blood, averaging 5.76 g/dl, also was lower than commonly seen human values; Siggaard-Andersen (5,8), for example,

used 7.2 g/dl in constructing a curve nomogram for human blood. Third, plasma and presumably erythrocytic bicarbonate concentrations are higher in porcine than in human arterial blood. Plasma bicarbonate averaged 31.6 mEq/l in this study as compared to the 22-26 mEq/l commonly seen in human plasma (16). The net effect of these species differences was a higher buffer base concentration in pig blood relative to human blood.

Buffer base concentration has been defined by Singer and Hastings (17) as the sum of bicarbonate and protein anion concentrations in whole blood, plasma, or erythrocytes. The buffer base alignment nomogram constructed by Singer and Hastings (17) would indicate a whole blood value of about 53 mEq/l for pigs, assuming a hematocrit value of 0.29 and a pH of 7.50. At a hematocrit value of 0.45 and a pH of 7.40, humans would have a whole blood buffer base value of about 47.5 mEq/l. It should be recognized that the Singer and Hastings nomogram (17) was designed for use with human blood and may not be applicable to swine blood. It assumes a plasma protein concentration of 7.2 g/dl, which is higher than the porcine value, and a blood temperature of 37 C, which is lower than the usual 38-39 C seen in pigs. The plasma buffer base concentration actually measured in the pigs of this study averaged 45.5 mEq/l, 31.6 mEq/l being attributable to bicarbonate and 13.9 mEq/l to albumin and globulin anions. Human plasma, assuming a pH of 7.40, a bicarbonate concentration of 24.5 mEq/l, and a protein concentration of 7.2 g/dl (17.4 mEq/l), would have a measured buffer base concentration of 41.4 mEq/l.

The plasma contribution to whole blood buffer base is determined, obviously, by the plasma fraction of whole blood and the plasma concentrations of bicarbonate and protein anions. For the pigs in the present study it was (1 - 0.29)X(31.6 + 13.9) or 32.3 mEq/l of blood, of which 22.4 mEq/l was contributed by bicarbonate and 9.9 mEq/l by plasma protein. For humans, a typical plasma contribution would be (1 - 0.45)X(24.5 + 17.4) or 23.1 mEq/l of blood, 13.5 mEq/l being attributable to bicarbonate and 9.6 mEq/l to plasma protein.

The erythrocyte contribution to whole blood buffer base is determined by the erythrocyte fraction of whole blood and the concentrations of protein (almost entirely hemoglobin) and bicarbonate. Of these, erythrocyte bicarbonate concentration is the most difficult to estimate since it depends on both intracellular pH and water content. Hemoglobin anion concentration calculated according to the procedure of Dill et al (18) would be 2.30 mEq/mM of hemoglobin for pig blood at a plasma pH of 7.50, and 2.06 mEq/mM of hemoglobin for human blood at a plasma pH of 7.40. If an average hemoglobin concentration of 20 mM/l of plasma-free erythrocytes is assumed (16,17), the hemoglobin contribution to blood buffer base would be 0.29 X 2.30 X 20, or about 13.3 mEq/l of blood in the pigs of this study, and about 0.45 X 2.06 X 20 or 18.5 mEq/l for a typical human. The erythrocytic bicarbonate contribution can be estimated as the difference between whole blood buffer base and the sum of the contributions of plasma

bicarbonate and protein and erythrocyte hemoglobin concentration. The porcine value would thus be 53-46.6 or 7.4 mEq/l and the typical human value 47.5-41.6 or 5.9 mEq/l of whole blood.

The differing buffer characteristics of porcine and human blood become more apparent, perhaps, if the foregoing calculations and estimates are summarized:

Buffer Base (mEq/l blood)

Blood Component	Pigs	Humans
Plasma protein	9.9	9.6
Plasma bicarbonate	22.4	13.5
Erythrocyte protein	13.3	18.5
Erythrocyte bicarbonate	7.4	5.9
Total	53.0	47.5

This summary shows that the higher buffer base concentration of porcine blood, compared to human blood, is attributable almost entirely to an elevated plasma bicarbonate concentration; the two species differ by about 9 mEq/l in this respect. Only hemoglobin anion concentration is higher in human than porcine blood, but this relative advantage is more than offset by differences in plasma bicarbonate concentration.

An elevated buffer base concentration along with an elevated pH also confers a greater capacity to compensate for an acid load on the part of the domestic pig. This difference between pigs and men was clearly evident when the effects of base excess changes on plasma pH and standard bicarbonate were compared (Table 4). For a given acid addition (base deficit) pig plasma, to maintain a P CO_2 of 40 torr, showed a similar bicarbonate change but a smaller pH change than human plasma and, because of higher values associated with zero base excess, the pig also showed higher equilibrium values for both variables. Base additions (base excess) to porcine plasma affected less of an increase in pH, but the higher values associated with zero base excess resulted in equilibrium values which were somewhat higher in porcine than in human plasma.

The accuracy and reliability of the data contained in this report obviously hinge upon the adequacy of the technical procedures used in the acid-base measurements and, in the evaluation of population characteristics, upon the physiologic status of the animals at the time of acid-base measurements. Insofar as could be determined, the Instrumentation Laboratory automated analyzer provided accurate measurements of blood pH and P CO_2 in the population study. Analyzer accuracy was assessed by determining the reproducibility of pH

determinations with buffer solutions of known hydrogen ion concentration and blood samples tonometered with known P CO_2 tensions. Similar pH reliability determinations were conducted with the Instrumentation Laboratory ultra-micro pH/blood gas system used to obtain data for nomogram construction. Accuracy and reproducibility of P CO_2 determinations with the automated analyzer were verified with blood samples tonometered against gases of known CO_2 tension. Bicarbonate concentrations computed by the automated analyzer were not consistent with pH-dependent pK' values for carbonic acid as determined by Severinghaus et al (13). Consequently, all bicarbonate concentrations in the population study were calculated from the Henderson-Hasselbalch equation using appropriate pK' values reported by Severinghaus et al (13).

From a physiological standpoint, the available literature contains few reports in which the acid-base status of swine was assessed under conditions comparable to those employed in the present study. Here, the population measurements were made under near-basal conditions. That is, the arterial samples were obtained from chronically-implanted catheters while the animals, after an overnight fast, had voluntarily assumed a recumbent position for at least one-half hour. The similarity of the various acid-base values when the measurements were repeated at hourly intervals (Table 2) indicates success in achieving metabolic and ventilatory steady states. The preponderance of porcine acid-base measurements reported in the literature was obtained from venous blood samples (see 19 for references). In most instances, little or no attention was given to the establishment of steady state conditions prior to blood sampling. Therefore, it was perhaps not too surprising to find wide divergence in the values reported by various investigators. Fewer attempts have been made to measure the acid-base characteristics of porcine arterial blood, and the reported data were based in most instances on blood samples which were obtained under abnormal or poorly-defined physiologic conditions. For instance, the reports by Lindberg (20), Fredlund et al (21), Becker et al (22), Lowery and Sugg (23), and Rokkanen et al (24) contain arterial data from anesthetized pigs subjected to various circulatory shock procedures; in some of these reports, data interpretation was complicated further by mechanical ventilation of the animals. Only two reports (4,25), insofar as can be determined, contain porcine arterial acid-base data measured in blood samples taken from unanesthetized animals by means of chronically-implanted catheters. One of these, by Van Den Hende et al (25), contained two pigs that were evaluated before and after a bout of treadmill running. Pre-run arterial blood values for pH were 7.43 and 7.49, P CO_2 42 and 40 torr, and base excess +2.5 and +7.0 mEq/l. The other study by Scott and McIntosh (4) contained 14 young pigs which had control arterial values which averaged 7.455 for pH, 44.1 torr for P CO_2, 30.8 mEq/l for bicarbonate, and +6.0 mEq/l for base excess. The high base excess values in both of these papers were attributable, presumably, to high bicarbonate concentrations and pH levels. Neither report (4,25) provided explicit information relevant to the metabolic or ventilatory status of the animals at the

acid-base measurement, hence divergence from the results obtained in the present study might be expected.

It should be recognized that the population acid-base characteristics and curve nomogram presented here may only be applicable to young domestic swine. Older animals may have different population characteristics which could alter the assignment criteria for zero base excess. In this regard, it should be noted that the plasma protein (26,27) and hemoglobin (28,29) concentrations of swine increase with age. Both of these variables could influence acid-base status.

CONCLUSIONS

Arterial blood obtained under near-basal conditions from immature domestic pigs has a higher pH, bicarbonate, and buffer base concentration than human blood similarly obtained. Porcine blood has a lower hemoglobin and plasma protein concentration and P O_2 than human blood, but the two species are similar in terms of arterial P CO_2.

The acid-base status of porcine blood samples cannot be determined accurately on the basis of nomograms or other procedures designed for use with human blood. A nomogram or other procedure specifically designed for porcine blood must be used.

RECOMMENDATIONS

Because use of a curve nomogram requires constant P CO_2 tonometry of blood samples, and such equipment often times is not readily available in many blood gas laboratories, an alignment nomogram should be constructed for porcine blood. Such a nomogram only requires the accurate measurement of pH and P CO_2 to assess acid base status, a task that is readly accomplished with all modern blood gas systems.

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Construction of a Base Excess Curve Nomogram for Porcine Blood

APPENDIX A

Construction of the porcine curve nomogram is based on the same general principles and procedures first reported in 1960 by Siggaard-Andersen and Engel (1) for human blood. The blood preparation and titration techniques are in accordance with a later technical revision, reported by Siggaard-Andersen in 1962 (2), which eliminated experimentally-induced errors in curve construction and, consequently, base excess estimation. The revised procedure also has been used by Emuakpor et al (3) to construct a curve nomogram for canine blood. In these reports, the pH of plasma and blood is assumed to be linearly related to log P CO_2 (4-7). The same assumption is made for swine blood. In addition, earlier reports defined zero base excess as existing when P CO_2 equaled 40 torr and pH equaled 7.40. This assumption is not made for porcine blood. Rather, zero base excess is defined for a P CO_2 of 40 torr, but a pH of 7.50. The latter is a porcine population characteristic. Finally, no attempt is made to construct a buffer base curve nomogram with porcine blood, since a buffer base alignment nomogram equivalent to that for human blood reported by Singer and Hastings (8) would be needed to obtain the requisite acid-base parameters needed for construction.

METHODS AND PROCEDURES

Equipment

1. Refrigerated clinical centrifuge, International Equipment Co., Model PR-2
2. Microhematocrit centrifuge, Lourdes Model MH
3. Tonometer (two), Instrumentation Laboratory, Model 237
4. Blood gas analyzer, Instrumentation Laboratory, Model 213-05
5. Hemophotometer, Fisher Model 740
6. Positive displacement micropipette, 3 ml adjustable, SMI Series G
7. Micropipette, 250 ul, Eppendorf
8. Buret, 25-ml
9. Buret, 10-ml (two)
10. Volumetric flask, 100-ml
11. Reagent bottles, glass-stoppered, 125-ml (ten)
12. Gas cylinder, reduction valve, 2-stage (two), Matheson Model 8

13. Vortex mixer, Scientific Industries

14. Test tube rack, Wasserman or Kahn

15. Stainless steel tray, 12 x 7-1/2 x 2 in

16. Flexible rule, 18-inch, K&E type 57-2817

17. Ruler, 12-inch

Supplies

1. 4% carbon dioxide in oxygen, compressed, Matheson primary standard grade, size 1A cylinder

2. 8% carbon dioxide in oxygen, compressed, Matheson primary standard grade, size 1A cylinder

3. Hydrochloric acid, 1 N, Fisher certified reagent grade, 1-liter bottle

4. Sodium bicarbonate, 1 N: dissolve 8.401 g reagent grade $NaHCO_3$ in 100 ml distilled water

5. Sodium chloride, 1 N: dissolve 5.844 g reagent grade NaCl in 100 ml distilled water.

6. Reference (calibration) buffer, pH 6.840, Instrumentation Laboratory

7. Reference (calibration) buffer, 7.384, Instrumentation Laboratory

8. Drabkins reagent, Fisher dry pack

9. Culture tubes, 12 x 75 mm, disposable

10. Centrifuge tubes, 50-ml, disposable, with caps

11. Pasteur pipettes, 7-inch, disposable

12. Spinal needle, 3-inch (four)

13. Microhematocrit tubes, heparinized

14. Heparin, 1000 units/ml, bottle

15. Pipette tips, plastic disposable (for SMI and Eppendorf micropipettes)

Preparation of Titration Reagents

All of the acid (HCl) and base ($NaHCO_3$) solutions used in the titration procedure must have a sodium concentration equivalent to that of plasma, namely 150 mEq/l, since ionic concentration can influence the acid-base characteristics of a buffer solution (2). To achieve this criterion in the nine acid and base solutions used in the titration procedure, the following preparation schedule is employed:

Solution	1 N HCl	1 N $NaHCO_3$	1 N NaCl	H_2O
1	10 ml	0 ml	15 ml	7.5 ml
2	7.5	0	15	77.5
3	5.0	0	15	80.0
4	2.5	0	15	82.5
5	0	0	15	85.0
6	0	2.5	12.5	85.0
7	0	5.0	10.0	85.0
8	0	10.0	5.0	85.0
9	0	15.0	0	85.0

All additions are delivered to a 100-ml volumetric flask. Additions of HCl and $NaHCO_3$ can be conveniently and accurately made from 10-ml burets and the NaCl from a 25-ml buret. Distilled water is added to bring the final volume to the 100-ml mark. When so prepared, the acid concentrations in solutions 1 through 5, respectively, are 100, 75, 50, 25, and 0 mEq/l, and the base concentrations in solutions 6 through 9, respectively, are 25, 50, 100, and 150 mEq/l. After preparation, the solutions are transferred to 125-ml reagent bottles for use in the titration procedure. Fresh solutions should be prepared every two weeks to minimize concentrating effects due to water loss from the reagent bottles or to base loss from the bicarbonate solutions due to absorption of atmospheric carbon dioxide.

Blood Collection and Preparation

About 60 ml of fresh blood is usually required for the complete titration procedure. In pigs, it can conveniently be obtained from a chronically implanted arterial or venous catheter, in humans from a convenient arm vein. Immediately after collection, the blood along with 1 ml of heparin (1000 units/ml) is placed in a 50-ml disposable plastic centrifuge tube, capped, and chilled to 0 C in a crushed ice and water bath. The hematocrit is determined in duplicate with a microhematocrit centrifuge and the blood is centrifuged at 2500 rpm for 20 minutes at 0 C. Plasma is then collected and placed in a separate 50-ml capped centrifuge tube maintained at 0 C. Twenty milliliters of plasma is needed for the complete titration procedure and the remainder is added to packed cells to achieve an erythrocyte-plasma mixture, i.e., blood, with a hematocrit of 0.5 to 0.6. After thorough mixing, the actual hematocrit is determined in triplicate with a

microhematocrit centrifuge. Hemoglobin concentration also is determined in triplicate with Drabkins reagent according to standard procedures described by the manufacturer of the hemophotometer used to measure concentration. Approximately 20 ml of blood is sufficient to complete the acid-base titration procedure. Both plasma and blood are kept in capped 50-ml centrifuge tubes at or near 0 C until used for tonometry.

Titration Procedure

While the plasma and blood are being prepared, two tonometers are brought to operating temperature, 38 C, and the gas flows to each are adjusted to approximately 250 ml/min. One tonometer receives a 4%, and the other an 8% CO_2 in O_2 mixture. While this is being accomplished, the blood gas analyzer also is brought to operating temperature, 38 C, and the pH electrode is calibrated with pH 6.840 and 7.384 reference buffers. Eighteen 12x75-mm culture tubes are then placed in a test tube rack and the latter is placed in a stainless steel tray containing crushed ice and water. Exactly 1 ml of blood is transferred to the bottom of each tube with an SMI positive displacement micropipette and an attached 18-gauge 3-inch spinal needle. Care should be exercised to assure homogeneity of the blood during the transfer process, i.e., erythrocyte sedimentation tends to occur when blood is left undisturbed. Upon completion of the transfer, all 18 tubes are lightly centrifuged (5 min at 2500 rpm) to obtain a plasma layer at the upper surface of the blood. This is done to minimize the potential adverse effects of subsequent acid and base additions on hemoglobin buffer characteristics (1,2). After centrifugation, the tubes are returned to the test tube rack and ice water tray. The tubes are used in pairs for the acid-base titration procedure. For each acid or base addition, one tube is used for tonometry with 4% CO_2 and the other for tonometry with 8% CO_2. The acid and base additions are made with a 250-ul Eppendorf pipette. This brings total fluid (blood plus acid or base) volumes to 1.25 ml and the concentration of added acid or base to one-fifth of that contained in the original reagent bottle. Final concentrations in the tube pairs, therefore, would be 20, 15, 10, 5, and 0 mEq/l of acid and 5, 10, 20, and 30 mEq/l of base. Additions are made at about 2.5-minute intervals, starting with the 100 mM HCl reagent. After each addition, the tube contents are rapidly stirred with a vortex mixer and transferred to the tonometer reaction vessel with a Pasteur pipette. The tonometer stirrer is activated, and equilibration with the CO_2 mixture is continued for 5 minutes. Immediately following equilibration, the blood sample is transferred directly from the tonometer to the blood gas apparatus by means of the capillary tube attached to the hydrogen ion electrode. After recording the pH value, the sample is discarded, a clean reaction vessel is placed in the tonometer, and the original vessel is cleaned with distilled water and dried by wiping with a clean 4x4 inch gauze sponge. With practice, the 2.5-minute interval between acid and base additions allows adequate time to complete all of the foregoing operations. When all blood

titrations are completed, the entire procedure is repeated with 1-ml plasma samples.

Nomogram Construction

Construction of the base excess curve nomogram progresses through three stages. The first involves use of the pH/log P CO_2 relationship to determine the pH value corresponding to a P CO_2 of 40 torr for each of the acid and base additions made to blood and plasma. The second stage involves plotting constant P CO_2 titration curves and determining the correction needed to meet the prescribed parameters for zero excess. The third stage involves the actual plotting of base excess loci which allow drawing of the curve nomogram. These stages are illustrated with typical data and computations acquired in constant P CO_2 titrations with porcine blood.

The pH/Log P CO_2 Relationship

As illustrated in Figure A1, three parallel P CO_2 lines were drawn from the ordinate of semilogarithmic graph paper--one representing the partial pressure for 4% CO_2, another for 8% CO_2, and the third a P CO_2 of 40 torr. Values for 4 and 8% CO_2 were obtained from the following equation:

$$P\ CO_2 = (B.P.-P\ H_2O)f$$

where B.P. refers to barometric pressure, P H_2O the partial pressure of water vapor in a saturated gas mixture at 38 C, and of the measured CO_2 fraction contained in the gas mixture. The 28.5 torr CO_2 line in Figure A1, for example, reflected a barometric pressure of 763 torr, a water vapor pressure of 49.7 torr, and a CO_2 fraction of 0.03992. Two such graphs were prepared, one for the blood samples and the other for the plasma samples which were tonometered against low and high CO_2 mixtures. On each graph, the point pairs are plotted for the pH values which were obtained after tonometry with each acid and base addition. These points were then connected with a straight line and pH values corresponding to a P CO_2 of 40 torr were determined.

Constant P CO_2 Titration Curves

The pH values obtained from titration at low and high P CO_2 and the extrapolated values for a P CO_2 of 40 torr were plotted on conventional graph paper as shown in Figure A2 for both plasma and blood. Curves representing the "best visual fit" were then drawn with a flexible rule. It is apparent from these curves that zero acid addition (i.e., 0.15 M NaCl) did not correspond to zero base excess as defined for porcine blood, i.e., when pH is 7.50 and P CO_2 is 40 torr. This lack of correspondence also has been seen in human (1,2) and dog (3) blood titrations and was due, presumably, to buffer dilution associated with the addition of acid and base before tonometry. To correct for this lack of correspondence, the zero base excess scale is

moved to the right as shown by the dotted lines and the revised X-axis scale in Figure A2, the latter may then be used to determine corrected base excess and pH values for the titration curves at constant low and high CO_2 tension (see dashed line indicating -20 mEq/l base excess in Figure A2).

Plotting the Base Excess Nomogram

Upon completion of the constant P CO_2 titration plots and determination of corrected base excess values for both blood and plasma, the requisite data became available for construction of the base excess curve nomogram. Semilogarithmic paper with parallel lines representing P CO_2 values corresponding to 40 torr and the tensions of the low and high CO_2 tonometry mixtures were drawn from the ordinate; pH values were placed on the abscissa. The loci needed to define the curve-nomogram were then plotted by the procedure illustrated in Figure A3. Accordingly, for a given blood base excess concentration (corrected) two pH values (corrected) were obtained from the low and high constant CO_2 tension curves and were placed at their appropriate positions on the semilogarithmic paper. A line connecting the two was then drawn. The intersection of these two lines defined the pH/P CO_2 coordinates for the particular base excess locus in the curve nomogram. The same procedure was followed in plotting all other loci, ranging from -20 to +20 mEq/l, included in the curve nomogram. Finally, the entire curve nomogram was defined by drawing a smooth line through all of the loci with a flexible rule. This nomogram depicted the base excess characteristics of blood and plasma from one particular pig. A composite curve based on titrations and curve plots of blood and plasma from many pigs was needed to depict porcine population characteristics. A standard bicarbonate (4-7) scale was added to the latter to enhance its usefulness as a tool to assess the acid-base status of pigs. In so doing, bicarbonate concentration should be calculated from the Henderson-Hasselbalch equation using the pH-related pK' values for carbonic acid dissociation reported by Severinghaus et al (9) and a CO_2 solubility coefficient of 0.0301.

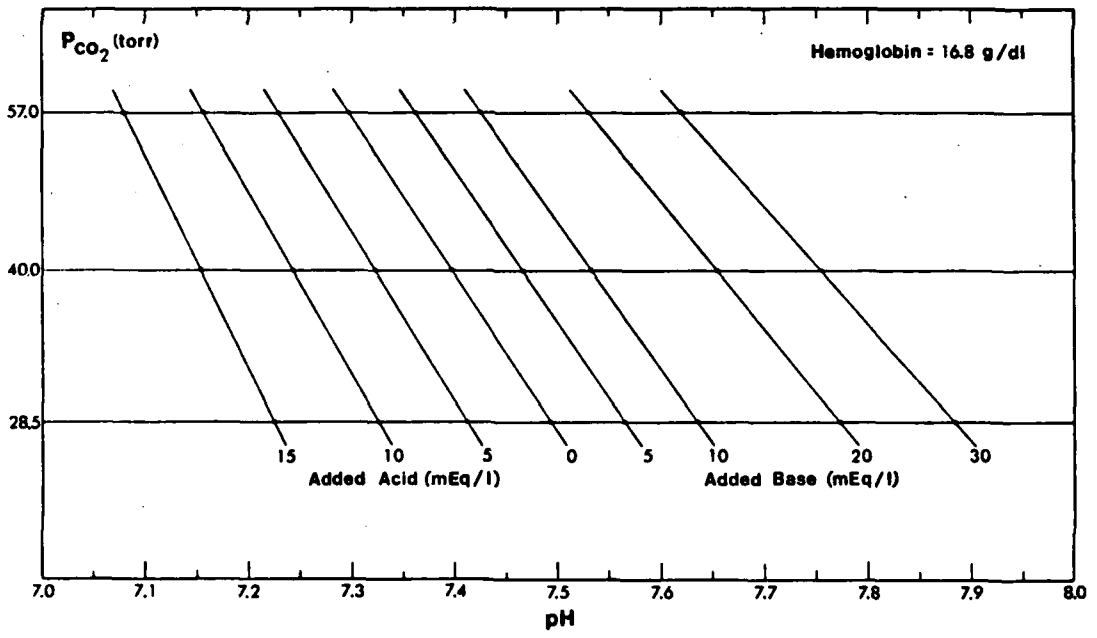

Figure A1. pH/log P CO_2 lines for sets of tonometry equilibrations at low (28.5 torr) and high (57.0 torr) CO_2 tensions of porcine blood at a hemoglobin concentration of 16.8 g/dl. Comparable plot also is made for plasma equilibrations.

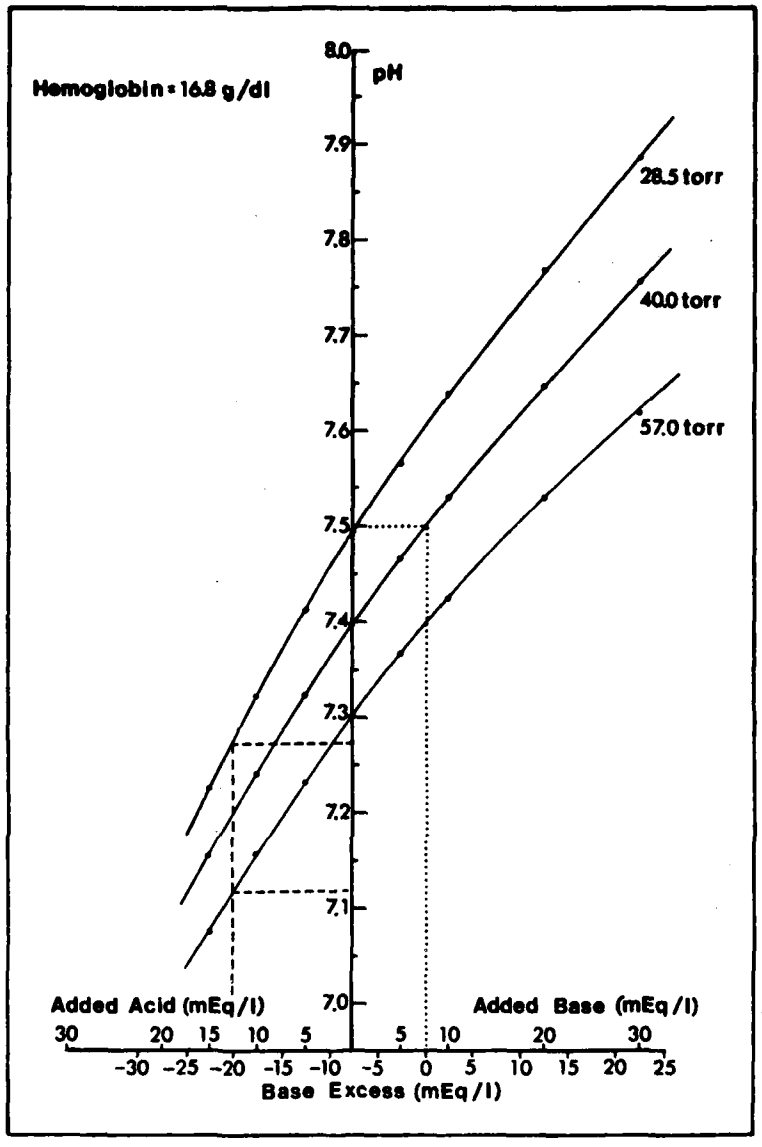

Figure A2. Constant P CO_2 titration plot for experimentally determined curves at a P CO_2 of 27.5 and 57 torr of blood (16.8 g/dl). Curve at 40 torr was determined by extrapolation (*Figure A1*). Base excess was defined as zero when pH was 7.50 and P CO_2 was 40 torr (dotted line). Dashed line depicts base excess (corrected) of 20 mEq/l.

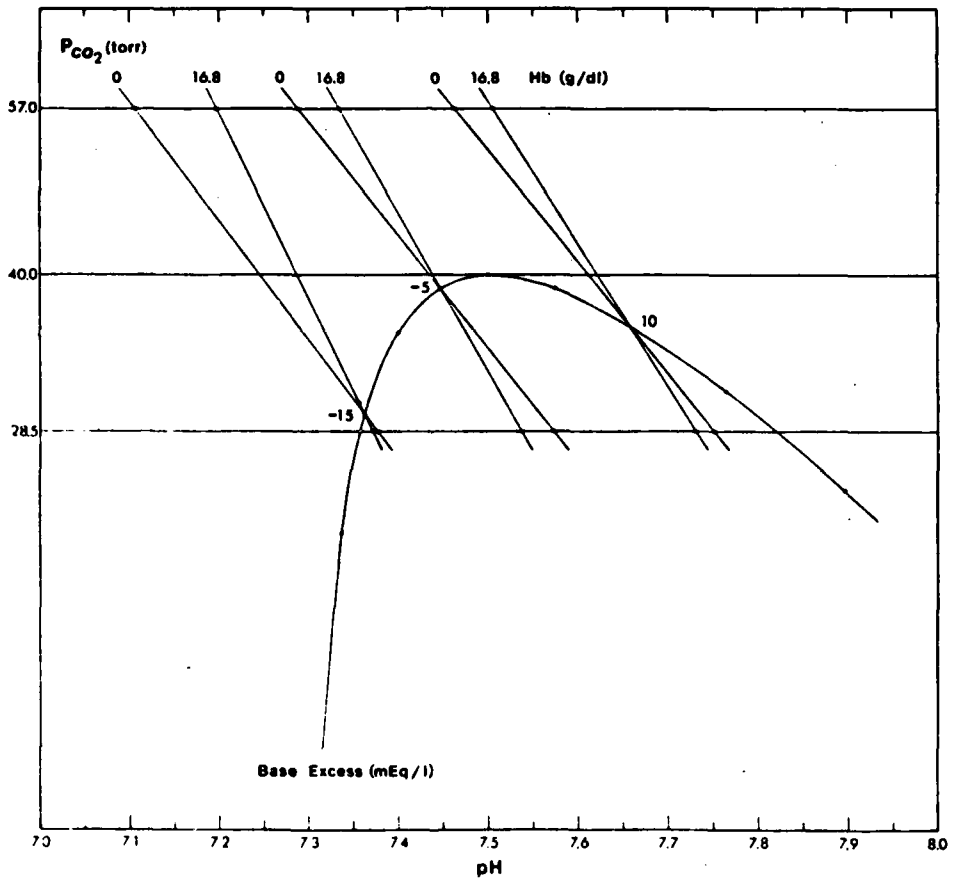

Figure A3. Technique for plotting blood base excess loci of −15, −10, and +10 mEq/l on the basis of pH/log P CO_2 coordinates obtained from constant P CO_2 titrations of plasma and blood (0 and 16.8 g Hb/dl, respectively).

COMMENT

Siggaard-Andersen and Engel (1), Siggaard-Andersen (2), and Emuakpor et al (3) have all used Radiometer equipment for the tonometry and pH measurements needed to construct a base excess curve nomogram. Here, Instrumentation Laboratory equipment was equally satisfactory if used properly. Experimental evaluation of the tonometer showed that both temperature and CO_2 equilibration could be achieved in less than two minutes but, to be on the safe side, a 5-minute period of tonometry was employed in constructing the porcine nomogram. The blood gas system was found to be accurate and reliable. Calibrations at the high and low end of the pH scale were maintained with deviations of less than 0.01 units over the 2-hour period required for plasma and blood tonometry; recalibration of the instrument was rarely needed during the course of tonometry.

Suitable substitutes could no doubt be found for most of the other equipment used in constructing the porcine curve nomogram. The sole exception to this generality is the pipette used to obtain aliquots of blood and plasma for tonometry. A variety of conventional glass and automatic pipettes were unsatisfactory. In most instances they delivered less volume than the calibration would indicate because of blood or plasma adherence to the pipette walls. The SMI positive displacement pipette was a far superior instrument if care was exercised in eliminating all bubbles during the pipetting procedure. When properly used, gravimetric tests and tests involving ^{51}Cr-labelled erythrocytes showed an accuracy of $\pm 2\%$ or less with the SMI Instrument.

The foregoing should not be taken as an indication that construction of a base excess curve nomogram was an easily accomplished task. On the contrary, it can be a most frustrating effort during the early stages. A variety of procedural and technical problems, some readily perceived but others not, can lead to inconsistencies in data collection. Pipetting accuracy is of paramount importance, and most problems in tonometry and subsequent curve plotting can be traced to inaccurate volume measurements. Another potential source of error involves the transfer of blood or plasma from the tonometer to the pH electrode. Exposure to air or other less readily appreciated variables, such as pressure changes associated with the transfer, can lead to inconsistent pH measurements. Finally, considerable practice in all aspects of the procedure is needed to assure consistency of the human element.

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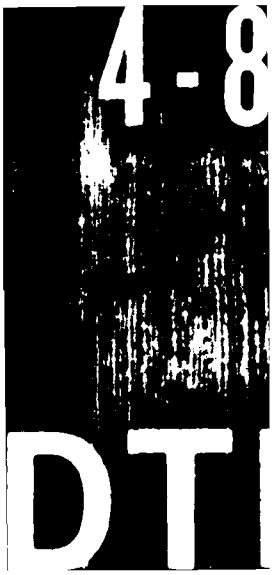
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